

Accession No. 410608-01

DATA EVALUATION RECORD

1. **CHEMICAL:** PCNB 2E.
Shaughnessey Number: Not available.
2. **TEST MATERIAL:** PCNB 2E; Lot No. 832001; 24% Active
Ingredient; an amber liquid.
3. **STUDY TYPE:** Freshwater Fish Flow-through Acute Toxicity
Test. Species Tested: Lepomis macrochirus.
4. **CITATION:** Bowman, J.H. 1989. Acute Flow-through Toxicity
of PCNB 2E to Bluegill sunfish (Lepomis macrochirus).
Prepared by Analytical Bio-Chemistry Laboratories, Inc.,
Columbia, Missouri. Report No. 36937. Submitted by AMVAC
Chemical Corporation, Los Angeles, California. Accession
No. 410608-01.
5. **REVIEWED BY:**

Kimberly Rhodes
Associate Scientist
KBN Engineering and
Applied Sciences, Inc.

Signature: *Kimberly Rhodes*
Date: *July 12, 1989*
6. **APPROVED BY:**

Prapimpan Kosalwat, Ph.D.
Staff Toxicologist
KBN Engineering and
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EEB Reviewer
Henry T. Craven
Supervisor, EEB/HED
USEPA

Signature: *P. Kosalwat*
Date: *July 12, 1989*

Susanne
Signature: *Henry T. Craven*
Date: *8/4/89*
7. **CONCLUSIONS:** This study appears scientifically sound and
fulfills the Guideline requirements for an acute 96-hour
flow-through toxicity test for a warmwater fish species.
The 96-hour LC50, based upon mean measured concentrations,
of PCNB to bluegill sunfish (Lepomis macrochirus) was 0.24
mg/L. Therefore, PCNB is classified as highly toxic to
bluegill sunfish. The NOEC was determined to be 0.057 mg/L
after 96 hours.
8. **RECOMMENDATIONS:** N/A



9. BACKGROUND:

10. DISCUSSION OF INDIVIDUAL TESTS: N/A

11. MATERIALS AND METHODS:

A. Test Animals: Bluegill sunfish (Lepomis macrochirus) were obtained from a commercial supplier in Missouri. The fish were maintained at the testing facility in well water and were fed newly hatched brine shrimp or a commercially available fish food daily. Seventy-two hours before the initiation of the test, bluegill sunfish were removed from the culture tank and placed in the temperature acclimation unit. During this time, the fish were held without food. The bluegill sunfish used as the control group during this study had a mean weight of 0.93 (\pm 0.31) grams and a mean length of 34 (\pm 3.3) millimeters at test termination. The biomass loading rate was 0.62 g/L. The laboratory environment was maintained on a 16-hour daylight photoperiod.

B. Test System: A proportional diluter system described by Mount and Brungs, utilizing a Hamilton Micro Lab 420 syringe dispenser, was used for the intermittent introduction of PCNB 2E test solutions and diluent water into each test chamber. The proportional diluter system used for the project was set to provide test levels approximately 50 percent dilutions of each other. The test aquaria were glass vessels filled to a depth of approximately 22 cm, with a width of 30.5 cm and a length of 50.2 cm. The diluter delivered one liter of test solution or control water to the test vessels at an average rate of 14 times per hour over the course of the study. This flow rate was sufficient to replace the 30-liter volume within the test chambers 11 times per day. Five concentrations of the test material with dilution water control were tested. The test chambers were immersed in a temperature controlled water bath held at $22 \pm 1^{\circ}\text{C}$.

Dilution water for the bluegill sunfish test was a blend of reverse osmosis water and ABC well water characterized as having a pH of 7.6 - 7.8, total hardness of 40 - 46 mg/L as CaCO_3 , total alkalinity of 48 - 56 mg/L CaCO_3 and specific conductance of 100 - 120 umhos/cm.

C. Dosage: 96-hour flow-through acute test.

D. Design: Based on the results of the preliminary testing, a control and five nominal PCNB 2E concentrations of 0.075, 0.15, 0.30, 0.60 and 1.2 mg/L were selected for definitive testing. Twenty bluegill sunfish were tested per concentration. All concentrations were observed once every 24 hours for mortality and abnormal effects. The water quality parameters (temperature, dissolved oxygen and pH) were measured in the control, low, middle and highest concentration containing surviving fish at 0, 48 and 96 hours of testing. Analytical samples were collected from each test level and the diluter stock at 0 and 96 hours of the exposure.

E. Statistics: The concentrations of toxicant lethal to 50% of the population (LC50's) and 95% confidence intervals were determined at 24-, 48-, 72-, and 96-hour exposure periods by the computer program developed by Stephan et al. (1978).

12. REPORTED RESULTS: The mean measured concentrations of PCNB 2E were 0.057, 0.073, 0.12, 0.26 and 0.58 mg/L. The mean measured concentrations ranged from 40% to 76% of the nominal concentrations. A white precipitate was noted in the diluter mixing cell which suggested that not all of the PCNB 2E was going into solution.

Mortality and behavioral observations during the acute toxicity test of PCNB 2E to bluegill sunfish are shown in Table 6 (attached). The 24-, 48-, 72-, and 96-hour LC50 values for PCNB 2E were >0.58, >0.58, 0.32 and 0.24 mg/L, respectively, based upon mean measured concentrations. The slope of the 96-hour dose-response line was 7.9. Behavioral/sublethal effects noted during the study included hemorrhaging, on-bottom orientation, loss of equilibrium, quiescence, light discoloration and surfacing. Given that mortality and/or behavioral/sublethal effects occurred at the test concentrations of 0.073, 0.12, 0.26 and 0.58 mg/L, a no-effect concentration of PCNB 2E toxicity to bluegill sunfish was determined to be 0.057 mg/L. This conclusion is supported by the lack of mortality or behavior/sublethal effects at 0.047 mg/L.

Water chemistry parameters measured at 0, 48 and 96 hours were within the specified limits for conducting aquatic toxicity tests. Temperature ranged from 21 to 22°C. Dissolved oxygen ranged from 7.9 to 8.6 mg/L, these values represented 94 and 102% saturation at 22°C, respectively. The pH ranged from 7.6 to 7.8.

13. STUDY AUTHOR'S CONCLUSIONS/QUALITY ASSURANCE MEASURES:

The 96-hour LC50 value for PCNB 2E was 0.24 mg/L with 95 percent confidence limits of 0.12 and 0.58 mg/L mean measured concentration. The NOEC (No-Observed-Effect Concentration) was 0.057 mg/L after 96 hours.

Quality Assurance and Good Laboratory Practice Regulation Statements were included in the report, indicating that the study was conducted in accordance with the FIFRA Good Laboratory Practice Standards set forth in 40 CFR Part 160.

14. REVIEWER'S DISCUSSION AND INTERPRETATION OF STUDY RESULTS:

A. Test Procedure: The test procedures were generally in accordance with protocols recommended by the Guidelines, but deviated from the SEP as follows:

- o The SEP recommends that fish be acclimated to study conditions for at least two weeks prior to testing. The bluegill sunfish were removed from the culture tank and placed in the temperature acclimation unit 72-hours before test initiation.

- o Six-hour temperature measurements were not recorded as required by the SEP for tests conducted in a water bath.

- o The SEP states that each designated treatment group should be exposed to a concentration of toxicant that is at least 60% of the next highest concentration. Each designated treatment group for the test was only 50% of the next highest concentration.

- o The SEP recommends a 16-hour light and an 8-hour dark photoperiod with a 15- to 30-minute transition period between light and dark. The report did not state whether a 15- to 30-minute transition period between light and dark was maintained.

B. Statistical Analysis: The reviewer used EPA's Toxanal computer program to calculate the LC50 values. These calculations are attached. The binomial test provides a 96-hour LC50 value of 0.24 mg/L with a 95 percent confidence interval of 0.12 to 0.58 mg/L which is the same as that reported by the author.

C. Discussion/Results: The study results appear to be scientifically valid. The 96-hour LC50 value, based upon mean measured PCNB 2E concentrations, was estimated to be 0.24 mg/L. Therefore, PCNB 2E is classified as highly toxic to bluegill sunfish (Lepomis macrochirus). The NOEC was determined to be 0.057 mg/L after 96 hours.

D. Adequacy of the Study:

(1) Classification: Core.

(2) Rationale: N/A

(3) Repairability: N/A

15. COMPLETION OF ONE-LINER FOR STUDY: Yes, 07-06-89.

TABLE 6

Mortality and Behavioral Observations During the Acute Toxicity Test of
PCNB 2E to Bluegill Sunfish (Lepomis macrochirus)

| Mean Measured Concentration mg/l (ppm) | No. Placed in Test | 24-Hour | | 48-Hour | | 72-Hour | | 96-Hour | |
|---|-----------------------------|---------|---|---------------|--|---------------|-----------------------------------|---------------|---------------------------------------|
| | | Mort. | Obs. | Cum. Mort. | Obs. | Cum. Mort. | Obs. | Cum. Mort. | Obs. |
| Control | 20 | 0 | 20 N | 0 | 20 N | 0 | 20 N | 0 | 20 N |
| 0.057 | 20 | 0 | 20 N | 0 | 20 N | 0 | 20 N | 0 | 20 N |
| 0.073 | 20 | 0 | 20 N | 0 | 2 HG 18 N | 0 | 3 HG 17 N | 0 | 3 HG 17 N |
| 0.12 | 20 | 0 | 20 N | 0 | 2 HG 18 N | 0 | 2 HG 18 N | 0 | 2 HG 2 LD; 16 N |
| 0.26 | 20 | 0 | 1 HG; 2 OB; 17 N | 0 | 1 HG 2 OB; 17 N | 5 | 1 OB/LOE/Q 1 LD; 10 SUR 3 N | 12 | 1 LOE/SUR 4 SUR; 2 N 1 LOE/OB/Q |
| 0.58 | 20 | 0 | 1 LOE/OB/Q; 5 LOE/Q; 3 SUR 1 LOE/Q/HG 6 OB/Q; 4 Q | 5 | 2 SUR/Q/LOE 1 LOE/Q; 2 Q/OB 1 HG/LOE/OB/Q 9 LOE/OB/Q | 20 | --- | 20 | --- |

Key to observations: N = Normal; HG = Hemorrhaging; OB = On Bottom Orientation; LOE = Loss of Equilibrium; Q = Quiescent; SUR = Surfacing; LD = Light Discoloration

TRADE SECRET

KIMBERLY RHODES PCNB 2E LEPOMIS MACROCHIRUS 07-06-89

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*****
CONC.      NUMBER      NUMBER      PERCENT      BINOMIAL
           EXPOSED     DEAD        DEAD        PROB. (PERCENT)
.58        20          20          100          9.536742E-05
.24        20          12          60.00001     25.17223
.12        20          0           0            9.536742E-05
.073       20          0           0            9.536742E-05
.057       20          0           0            9.536742E-05
*****
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THE BINOMIAL TEST SHOWS THAT .12 AND .58 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS .236161

WHEN THERE ARE LESS THAN TWO CONCENTRATIONS AT WHICH THE
PERCENT DEAD IS BETWEEN 0 AND 100, NEITHER THE MOVING AVERAGE
NOR THE PROBIT METHOD CAN GIVE ANY STATISTICALLY SOUND RESULTS.

Shaughnessy No. Not availableChemical Name PCNB 2E

Chemical Class _____

Page _____ of _____

Study/Species/Lab/
AccessionChemical
% a.l.

Results

Reviewer/
DateValid
Stat14-Day Single Dose Oral LD₅₀LD₅₀ = mg/kg (95% C.L.) Contr. Mort.(X) =

Species _____

Slope = # Animals/Level = Age(Days) =

Sex =

Lab _____

14-Day Dose Level mg/kg/(X Mortality)
() , () , () , () , () , () , () , ()

Acc. _____

Comments:

14-Day Single Dose Oral LD₅₀LD₅₀ = mg/kg. (95% C.L.) Contr. Mort.(X) =

Species _____

Slope = # Animals/Level = Age(Days) =

Sex =

Lab _____

14-Day Dose Level mg/kg/(X Mortality)
() , () , () , () , () , () , () , ()

Acc. _____

Comments:

8-Day Dietary LC₅₀LC₅₀ = ppm (95% C.L.) Contr. Mort.(X) =

Species _____

Slope = # Animals/Level = Age(Days) =

Sex =

Lab _____

8-Day Dose Level ppm/(X Mortality)
() , () , () , () , () , () , () , ()

Acc. _____

Comments:

8-Day Dietary LC₅₀LC₅₀ = ppm (95% C.L.) Contr. Mort.(X) =

Species _____

Slope = # Animals/Level = Age(Days) =

Sex =

Lab _____

8-Day Dose Level ppm/(X Mortality)
() , () , () , () , () , () , () , ()

Acc. _____

Comments:

48-Hour LC₅₀LC₅₀ = PP (95% C.L.) Contr. Mort.(X) =

Species _____

Slope = # Animals/Level = Sol. Contr. Mort.(X) =

Temperature =

Lab _____

48-Hour Dose Level pp/(X Mortality)
() , () , () , () , () , () , () , ()

Acc. _____

Comments:

96-Hour LC₅₀LC₅₀ = 0.24 ppm (95% C.L.) Contr. Mort.(X) = 0Species Lepomis macrochirus

Slope = N/A # Animals/Level = 20 Sol. Contr. Mort.(X) = N/A

Temp. = 22 ± 1 °C

Lab Analytical Bio-Chemistry
Laboratories, Inc.

Acc. 410608-01

96-Hour Dose Level ppm/(X Mortality)
0.057 | 0.1 | 0.073 | 0.1 | 0.12 | 0.1 | 0.26 | 60 | 0.56 | 100

Comments: Based on mean measured concentrations.

96-Hour LC₅₀LC₅₀ = PP (95% C.L.) Contr. Mort.(X) =

Species _____

Slope = # Animals/Level = Sol. Contr. Mort.(X) =

Temp. =

Lab _____

96-Hour Dose Level pp/(X Mortality)
() , () , () , () , () , () , () , ()

Acc. _____

Comments:

K. R.
7/6/09 Core